

-- Further, a full-length cDNA cloning of human VRL-2 was performed. To identify start codon of VRL-2, two primers (5'-GCT GTC GGG GAA GAG GCG GGC ACA CTT G-3' (SEQ ID NO:5) and 5'-GCA GCA GTT CAT TGA TGG GCT CCA CAG C (SEQ ID NO:6)) designed from the sequence data of Incyte clone and human pancreas Marathon-Ready™ cDNA (Clontech, Palo Alto, Calif.) were used. 5' rapid amplification of cDNA ends (5'RACE) was performed under the following conditions: 94°C for 1 min and 68°C for 2 min, 30 cycles. The amplified 1.1 Kb RACE product were cloned and sequenced by automated DNA sequencer ABI PRISM 310 (PE Biosystems, Foster City, Calif.) produced by TOYOBO. These sequence data showed that 5'RACE products contained two kinds of 5' portion that possess a potential initiator codon downstream of an in-frame termination codon. VRL-2 constituted a subfamily that contained at least two spliced variants. One clone was designated as VRL-2a, and the other was designated as VRL-2b.

a' To isolate complete open reading frame of VRL2a and VRL-2b, PCR was performed using Advantage-HF PCR Kit (Clontech, Palo Alto, Calif.) and human kidney cDNA library (Clontech, Palo Alto, Calif.). Two sets of primers were designed from sequence data of 5'RACE products and Incyte clone; VRL-2a Forward 5'-ATC TGC GCA TGA AGTTCC AG-3' (SEQ ID NO:7), VRL-2b Forward 5'-GAC ATC GCG GAG CGC ACC GGC AAC ATG-3' (SEQ ID NO:8) and VRL-2a,b Reverse 5'-GCT GGA CTA GAA ATG AGT GGG CAG AGA A (SEQ ID NO:9). PCR was performed under the following conditions: 94°C for 20 sec, 58°C for 30 sec and 72°C for 30 sec, 25 cycles. PCR products of 2.6 kb (VRL-2a) and 1.9 kb (VRL-2b) were cloned and sequenced by automated DNA sequencer ABI PRISM 310 (PE Biosystems, Foster City, Calif.; produced by TOYOBO Gene Analysis. The amino acid sequences of VRL-2a and VRL-2b are shown in SEQ ID NO:2 and SEQ ID NO:4, respectively.--

This amendment is made to comply with the 37 C.F.R. § 1.821(d) and no new matter is added by such amendment.